Bioorganic & Medicinal Chemistry 19 (2011) 763–770

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Design, synthesis and evaluation of novel tacrine-multialkoxybenzene hybrids as dual inhibitors for cholinesterases and amyloid beta aggregation

Wen Luo, Yan-Ping Li, Yan He, Shi-Liang Huang, Jia-Heng Tan, Tian-Miao Ou, Ding Li, Lian-Quan Gu, Zhi-Shu Huang *

School of Pharmaceutical Sciences, Sun Yat-sen University, Guangzhou 510006, People's Republic of China

ARTICLE INFO

Article history:
Received 12 November 2010
Revised 5 December 2010
Accepted 6 December 2010
Available online 13 December 2010

Keywords:
Tacrine hybrids
Anti-Alzheimer agent
Acetylcholinesterase
Butyrylcholinesterase
Self-induced Aβ aggregation

ABSTRACT

A new series of tacrine-multialkoxybenzene hybrids (**9a**–**9n**) were designed, synthesized and evaluated as dual inhibitors of cholinesterases (ChEs) and self-induced β-amyloid (Aβ) aggregation. All the synthesized compounds had high acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE) inhibitory activity with IC_{50} values at the nanomolar range, which were much better than tacrine alone. A Lineweaver–Burk plot and molecular modeling study showed that these hybrids targeted both the catalytic active site (CAS) and the peripheral anionic site (PAS) of AChE. Besides, compounds **9a**–**9f** with methylenedioxybenzene moiety showed higher self-induced Aβ aggregation inhibitory activity than a reference compound, curcumin. These compounds could be selected as multi-potent agents for further investigation to treat AD.

1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative brain disorder that is characterized by dementia, cognitive impairment, and memory loss.[1,2] Although many factors have been implicated in AD, its etiology is not completely known. There are diverse hallmarks such as low levels of acetylcholine, β-amyloid (Aβ) deposits and τ-protein aggregation, which seem to play significant roles in the disease.[3–5]

Current treatment of AD focuses on increasing cholinergic neurotransmission in the brain through inhibiting cholinesterases (ChEs) activity with drugs including tacrine, donepezil, rivastigmine and galantamine (Fig. 1). But clinical experience has shown that ChEs inhibition alone is a palliative treatment, which does not address AD's etiology.[6] Due to the multi-pathogenesis of AD, one of the current strategies is to develop novel anti-Alzheimer agents with multiple potencies,[7] including compounds with inhibitory activity for both ChEs and Aβ self-aggregation, and the strategy of combining these two properties in one structure was confirmed to be successful.[8]

Two types of cholinesterase (ChE) were found in the central nervous system, including acetylcholinesterase (AChE) and butyrylcholinesterase (BuChE). AChE plays a crucial role in central and peripheral nervous systems. Control of the AChE enzyme activity can be used for the treatment of diseases associated with acetylcholine depletion, such as Alzheimer's disease.[9] On the other hand, BuChE has been recently considered as a potential target because it also plays an important role in regulating acetylcholine level.[10] Therefore, the concurrent inhibition of both AChE and BuChE should provide additional benefits in the treatment of AD.

The progressive deposition of Aβ in the brain of AD patients is generally considered to be fundamental to the development of neurodegenerative pathology. The cell toxicity associated with Aβ fibril aggregation provides an explanation for the neuronal cell loss found in AD patients.[11,12] Therefore, Aβ fibril aggregation in the brain is currently another potential target for the treatment of AD.[13,14] Hence, several series of inhibitors including curcumin[15,16] and benzofuran analogues[17,18] have been developed, and found to interfere with the self-induced aggregation of Aβ.

Tacrine was the first approved ChEs inhibitor by the FDA for the treatment of AD, although its side effects, the search for tacrine hybrids is still of interest. Recent years, many of studies focused on the combined effects of ChEs inhibition for the enhancement of the cholinergic neurotransmission and reduction of the Aβ fibril self-aggregation by conjugating tacrine with other active groups. Based on this strategy, many of tacrine hybrids (**A** and **B** in Fig. 1) were synthesised.[19,20]

Our research group has been involved in the development of ChEs inhibitors as potential drugs of AD for many years.[21–23] Recently, in our routine screening for compounds with inhibitory activity towards Aβ aggregation, it was found that compounds

* Corresponding author. Tel./fax: +86 20 39943056.
E-mail address: ceshzs@mail.sysu.edu.cn (Z.-S. Huang).

0968-0896/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2010.12.022

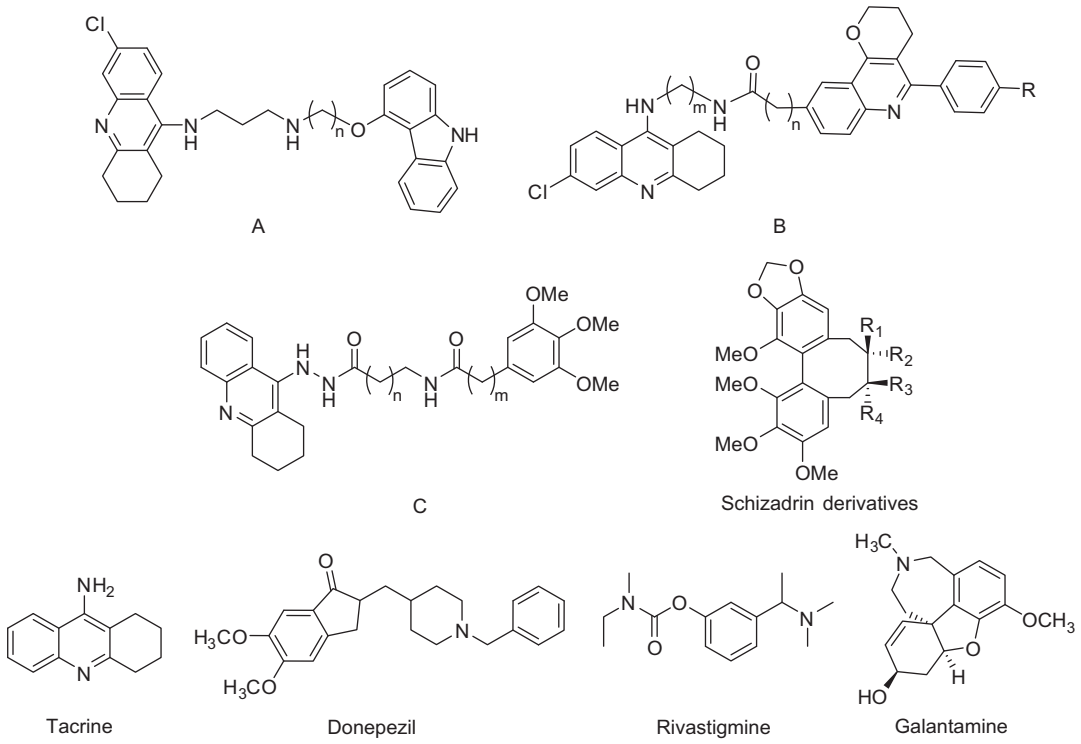


Figure 1. Chemical structures of tacrine, donepezil, rivastigmine, galantamine, schizadrin derivatives, and tacrine hybrids.

containing a methylenedioxybenzene moiety had significant activity for inhibiting Aβ self-induced aggregation. In addition, it has been reported that the schizadrin derivatives (Fig. 1) with a methylenedioxybenzene group show significant inhibition for the activity of AChE, and the methylenedioxybenzene group is important through the structure–activity relationship studies.[24] Moreover, some studies have suggested that electron-rich aromatics would bind to the peripheral binding site (**C** in Fig. 1), which have been confirmed in a comparison of aromatics with differing electron density.[25–27] Therefore, in the present study, in order to further explore the anti-Alzheimer potential of the electron-rich aromatics, a series of new inhibitors aimed at both ChEs and Aβ self-induced aggregation were designed by conjugating the methylenedioxybenzene or di- (or tri-) methoxybenzene moiety with tacrine using a long chain linker. It is possible that the multialkoxybenzene inhibit AChE activity through binding with PAS of AChE and block Aβ self-aggregation.

2. Results and discussion

2.1. Chemistry

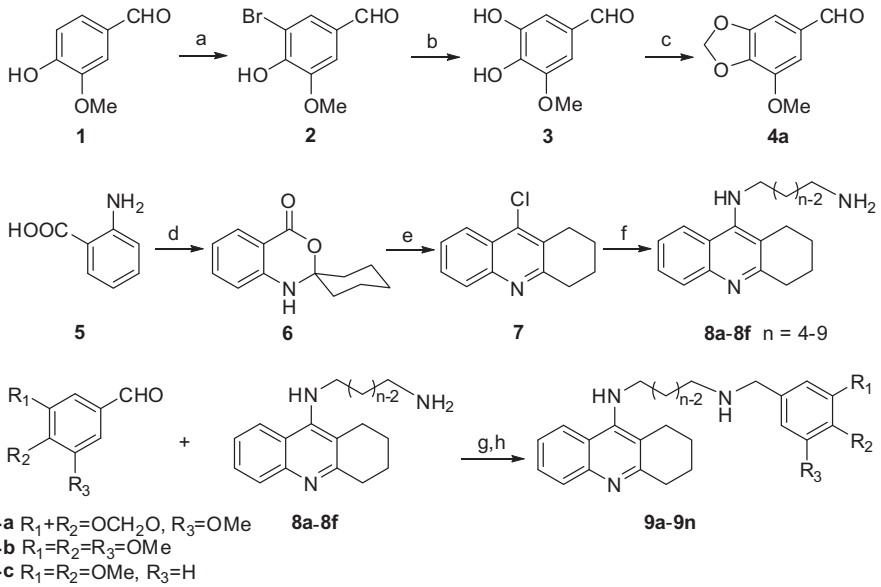
Fourteen tacrine-multialkoxybenzene hybrids (**9a**–**9n**) with linkers of varying chain length were prepared as shown in Scheme 1. Vanillin (**1**) was treated with bromine in CH_3COOH to generate 5-bromovanillin (**2**), which was heated with NaOH and copper powder in water to give 3,4-dihydroxy-5-methoxybenzaldehyde (**3**). Methylenation of compound **3** with a reported procedure afforded the aldehyde **4a** in a yield of 72%.[28] Compound **7** was synthesized from anthranilic acid (**5**) in 90% overall yield using a previously reported method.[29] Then, reaction of compound **7** with diaminoalkyl derivatives under reflux for 18 h in 1-pentanol gave the intermediates 9-alkylaminotetrahydroacridines (**8a**–**8f**) in good yields (60–70%). This step was carried out in the presence of catalytic amount of KI. The final products, compounds **9a–9n** were obtained by the reaction of multialkoxy benzaldehyde **4a**–**4c** with intermediates **8a**–**8f** followed with reduction using $NaBH_4$ in MeOH.[30]

2.2. In vitro inhibition studies on AChE and BuChE

To determine the potential of the target compounds **9a–9n** for the treatment of AD, their ChEs inhibitory activity was determined by the method of Ellman et al.[31] The IC_{50} values for AChE and BuChE inhibitions were summarized as shown in Table 1. The results showed that all the tested compounds (**9a**–**9n**) had significant ChEs inhibitory activity and 1–11.5-fold of inhibition selectivity for BuChE over AChE. Compound **9e**, with a methylenedioxybenzene group, showed the most potent inhibition for AChE with an IC_{50} value of 7.98 nM. Compound **9g**, with trimethoxy substituted benzene moiety, exhibited the strongest inhibition to BuChE with an IC_{50} value of 2.59 nM.

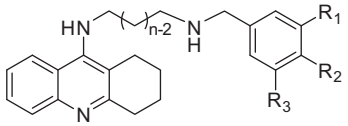
With the same chain length, the inhibition activity of methylenedioxybenzene hybrids **9c**–**9f** for AChE was slightly better than that of trimethoxybenzene and dimethoxybenzene hybrids **9g**–**9n**. In contrast, the inhibition activity of **9c**–**9f** for BuChE was slightly weaker than that of **9g**–**9n**. Compounds **9g**–**9j** with three methoxy groups on benzene ring were best inhibitors for BuChE. It is reasonable that the binding pockets of BuChE is bigger than that of AChE and this could allow bulky compounds to better fit inside the gorge of BuChE.

The optimal chain length between two aromatic units determined experimentally for inhibiting AChE and BuChE were eight (**9e**, **9i**, **9m**) and six (**9c**, **9g, 9k**) methylene groups, respectively. The variation of chain length for the inhibitors had more influence on their inhibition to AChE rather than BuChE. The possible reason is conformational differences between these two enzymes. BuChE does not have a functional peripheral site, and the active site of BuChE is wider than that of AChE. Therefore, BuChE had less restriction to inhibitors with varying linker length.



Scheme 1. Synthesis of tacrine-multialkoxybenzene hybrids. Reagents and conditions: (a) Br_2, AcOH, rt; (b) NaOH, Cu, H_2O, reflux; (c) K_2CO_3, CH_2Br_2, CuO, DMF, 140 °C; (d) cyclohexanone, toluene, reflux; (e) $POCl_3$, 120 °C; (f) diamine, KI, 1-pentanol, 160 °C; (g) methanol, rt; (h) $NaBH_4$, methanol, rt.

Table 1
Inhibition of ChE activity, selectivity index and inhibition of Aβ(1–42) self-induced aggregation



Compds	*n*	Structure of methylenedioxy or methoxyphenyl group	IC_{50}[a] for AChE (nM)	IC_{50}[b] for BuChE (nM)	Selectivity index[c]	Aβ(1–42) aggregation inhibition[d] (%)
9a	4	$R_1 + R_2 = OCH_2O$; R_3 = OMe	113.02 ± 1.67	46.29 ± 3.2	2.4	63.81 ± 3.22
9b	5	$R_1 + R_2 = OCH_2O$; R_3 = OMe	78.55 ± 0.28	29.83 ± 0.03	2.6	63.10 ± 13.65
9c	6	$R_1 + R_2 = OCH_2O$; R_3 = OMe	20.52 ± 0.03	5.19 ± 0.20	4.0	67.13 ± 5.57
9d	7	$R_1 + R_2 = OCH_2O$; R_3 = OMe	18.43 ± 0.15	9.73 ± 0.06	1.9	65.01 ± 9.85
9e	8	$R_1 + R_2 = OCH_2O$; R_3 = OMe	7.98 ± 0.12	7.94 ± 0.02	1.0	65.16 ± 1.84
9f	9	$R_1 + R_2 = OCH_2O$; R_3 = OMe	18.66 ± 0.17	11.36 ± 0.21	1.6	68.49 ± 3.63
9g	6	$R_1 = R_2 = R_3$ = OMe	22.41 ± 0.21	2.59 ± 0.14	8.7	—
9h	7	$R_1 = R_2 = R_3$ = OMe	17.29 ± 1.15	3.55 ± 0.15	4.9	—
9i	8	$R_1 = R_2 = R_3$ = OMe	9.77 ± 0.49	3.38 ± 0.25	2.9	—
9j	9	$R_1 = R_2 = R_3$ = OMe	20.19 ± 0.20	4.73 ± 0.36	4.3	—
9k	6	$R_1 = R_2$ = OMe; R_3 = H	30.92 ± 2.72	2.68 ± 0.17	11.5	—
9l	7	$R_1 = R_2$ = OMe; R_3 = H	24.03 ± 0.33	5.51 ± 0.10	4.4	—
9m	8	$R_1 = R_2$ = OMe; R_3 = H	11.35 ± 0.19	5.57 ± 0.83	2.0	—
9n	9	$R_1 = R_2$ = OMe; R_3 = H	25.10 ± 2.96	5.67 ± 0.32	4.4	—
Tacrine	—	—	192.6 ± 2.0	27.08 ± 1.16	7.1	—
Curcumin	—	—	—	—	—	51.77 ± 3.33

[a] AChE from *electric eel*; IC_{50}, inhibitor concentration (means ± SEM of three experiments) for 50% inactivation of AChE.
[b] BuChE from *equine serum*; IC_{50}, inhibitor concentration (means ± SEM of three experiments) for 50% inactivation of BuChE.
[c] Selectivity index = IC_{50} (AChE)/IC_{50} (BuChE).
[d] Inhibition of self-mediated Aβ(1–42) aggregation, the thioflavin-T fluorescence method was used, the mean ± SD of at least three independent experiments and the measurements were carried out in the presence of 20 μM compounds.

2.3. Kinetic characterization of AChE inhibition

The inhibition of AChE by a representative compound **9e** was further investigated using graphical analysis of steady state inhibition data as shown in Figure 2. The Lineweaver–Burk plots showed both increasing slopes and increasing intercepts for higher inhibitor concentration. The pattern indicated a mixed-type inhibition, which was similar to that of tacrine. This result showed that compound **9e** was able to bind both CAS and PAS of AChE which was also in agreement with the results of molecular modeling studies.

2.4. Molecular modeling study

With the aim of obtaining useful information about the binding interactions between compound **9e** and *Tc*AChE (PDB code: 1ACJ), a molecular modeling study was performed using the docking program AUTODOCK 4.0 package with PyMOL program as shown in Figure 3.[32,33]

The docking result demonstrated that all of the compounds exhibited multiple binding modes with AChE. In the **9e**–*Tc*AChE complex, compound **9e** occupied the entire enzymatic CAS,

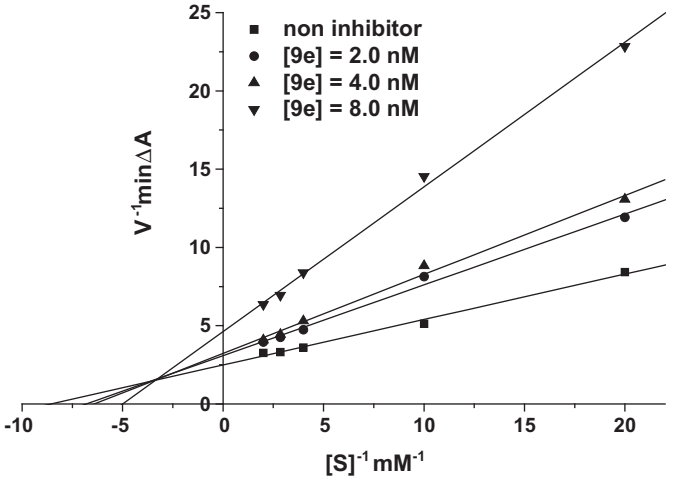


Figure 2. Lineweaver–Burk plot for the inhibition of acetylcholinesterase by compound **9e**.

mid-gorge and PAS. The tacrine moiety was bound to CAS, displaying a classic π–π stacking interaction between Trp84 and Phe330, with the ring-to-ring distance being 3.4 Å and 3.8 Å, and the protonated nitrogen atom of the quinoline ring establishes a hydrogen bond (3.4 Å) with the carbonyl group of the main chain of His440. At the PAS, the methylenedioxybenzene stacked against the Trp279 through π–π stacking with the distance of 3.8 Å. Similar interactions were found in **9e** in complex with *Hu*BuChE (PDB code: 1POI).

2.5. Inhibition of self-mediated Aβ(1–42) aggregation

The derivatives **9a–9f** were tested for their ability to inhibit self-mediated aggregation of Aβ(1–42) by using a thioflavin T fluorescence method.[34] Compared with the reference compound curcumin, the results showed that compounds **9a–9f** with methylenedioxy group apparently prevented the self-mediated Aβ aggregation with percentages of inhibition ranging from 63% to 68%, which were higher than that of curcumin (51%) (Table 1 and Fig. 4). The most potent compounds were **9c** and **9f**, and the inhibitory potency did not depend on the chain length of the connecting linker. Compounds **9g** and **9k** have the same chain length with compound **9c**, their activity (data not shown) were lower than that of curcumin. The current data are not sufficient to establish a structure-activity relationship for the activity toward Aβ aggregation, and further investigation is required.

3. Conclusion

In summary, six new tacrine-methylenedioxybenzene hybrids **9a–9f**, and eight new tacrine-trimethoxybenzene and tacrine-dimethoxybenzene hybrids **9g–9n** were synthesized and subjected to biological evaluation. The results showed that these synthetic compounds had high ChEs inhibitory potency and good selectivity for BuChE over AChE, which were similar to or better than those of tacrine. The methylenedioxy substituted hybrids (**9c–9f**) showed higher inhibitory effects on AChE, and compound **9e** with eight methylene groups had best AChE inhibitory activity. The inhibition kinetics of **9e** was analyzed using Lineweaver–Burk plots, which revealed that the compound was a mixed-type inhibitor, and could bind to both the CAS and PAS of AChE which was also in agreement with the results of molecular modeling studies. In addition, **9a–9f** exhibited higher self-induced Aβ aggregation activity than curcumin. Our above results should shed light on the design and development of new multi-potent anti-AD agents.

4. Experimental section

4.1. Chemistry

^{1}H and ^{13}C NMR spectra were recorded using TMS as the internal standard in $CDCl_3$ with a Bruker BioSpin GmbH spectrometer at 400 MHz and 100 MHz, respectively. MS spectra were recorded on a Shimadzu LCMS-2010A instrument with an ESI mass selective detector. High resolution mass spectra (HRMS) were recorded on Shimadzu LCMS-IT-TOF. Flash column chromatography was performed with silica gel (200–300 mesh) purchased from Qingdao Haiyang Chemical Co. Ltd. The purities of synthesized compounds were confirmed to be higher than 95% by analytical HPLC performed with a dual pump Shimadzu LC-20AB system equipped with a Ultimate XB-C18 column (4.6 × 250 mm, 5 μm) and eluted with methanol/water (35:65–45:55) containing 0.1% TFA at a flow rate of 0.5 mL/min. Melting points (mp) were determined using an SRS-OptiMelt automated melting point instrument without correction.

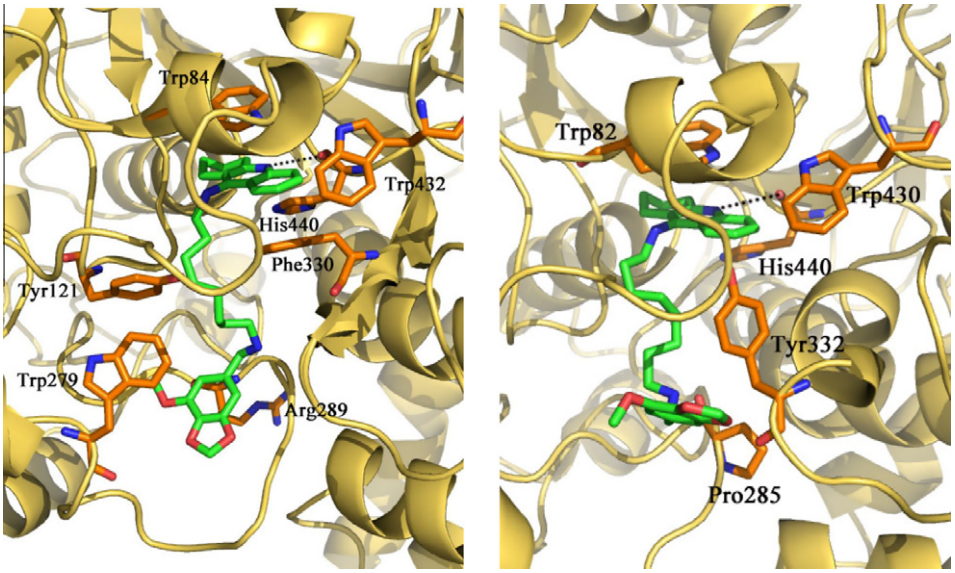


Figure 3. Docking models of compound-enzyme complex. Representations of compound **9e** interacting with residues in the binding site of *Tc*AChE and *Hu*BuChE. The compounds are rendered in green stick models, and the residues are rendered in orange sticks. Pictures are created with PyMOL.

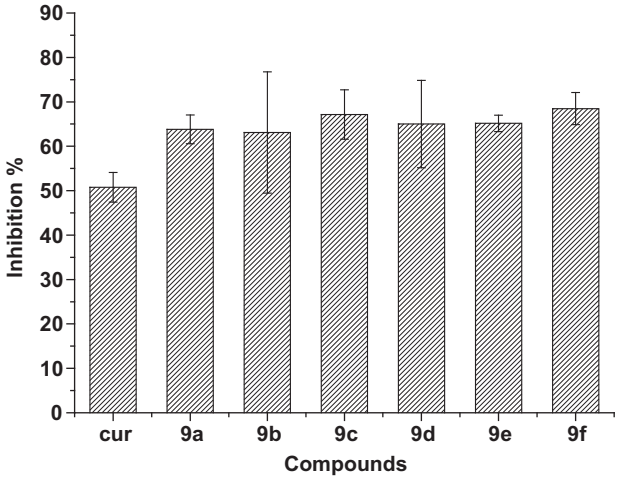


Figure 4. Inhibition of self-mediated Aβ(1-42) aggregation by compounds **9a–9f** comparing with that of curcumin. The thioflavin-T fluorescence method was used, and the measurements were carried out in the presence 20 μM compounds. The mean ± SD values from three independent experiments were shown.

4.2. Synthesis of intermediate 4

Aldehyde **4b** and **4c** are available commercially. Compound **3** was synthesized from vanillin **1** which was reported in many papers. Intermediate **4a** was prepared in the procedure described below.

CuO (0.13 g), anhydrous K_2CO_3 (1.75 g) and CH_2Br_2 (1.25 g) were added to a solution of compound **3** (1.0 g) in anhydrous DMF (12.5 ml), and the mixture was heated at 140 °C for 4 h. The reaction mixture was poured into water, and the product was extracted with ether. The ethereal extract was washed successively with 2% HCl, 2% NaOH and water. After drying over anhydrous K_2CO_3 and evaporating the solvent, light brown crystalline mass was obtained, which on recrystallization from MeOH, afforded colorless pillars. MS (ESI) *m/z*: 181.1 $(M+H)^+$. Yield: 72%, mp 129–130 °C (lit.[35] mp 131–132 °C).

4.3. General procedures for the preparation of intermediate 8a–8f

9-Chloro-1,2,3,4-tetrahy-droacridine **7** (0.44 g, 2 mmol), diamine (12 mmol), catalytic amount KI (0.05 g) and 1-pentanol (5 mL) were combined and heated to reflux (160 °C) for 10 h. After cooling to room temperature, the mixture was diluted with CH_2Cl_2 (50 mL) and then washed with 10% NaOH (1 × 50 mL) and water (2 × 40 mL). The organic layer was dried over $MgSO_4$, filtered, concentrated in vacuo, and purified by flash column chromatography with $CHCl_3$/MeOH/NH_4OH (5:1:0.5%) elution.

4.4. General procedures for the preparation of compound 9a–9n

Aromatic aldehydes **4** (1 mmol) and intermediate **8a–8f** (1 mmol) were stirred in MeOH for 4 h and then were reduced directly by $NaBH_4$ (4 mmol) at room temperature for 4 h. The solvent was evaporated and the residue was poured into water and extracted with EtOAc, the solution was dried over $MgSO_4$ and then concentrated, the compounds were purified by flash chromatography with $CHCl_3$/MeOH/NH_4OH (30:1:0.5%) elution.

4.4.1. N^1-((7-Methoxybenzo[*d*][1,3]dioxol-5-yl)methyl)-N^4-(1,2,3,4-tetrahydroacridin-9-yl)butane-1,4-diamine (9a)

Intermediate **4a** was treated with **8a** according to general procedure to give the desired product **9a** as light yellow oil (76% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (d, J = 8.5 Hz, 1H), 7.90 (d, J = 8.4 Hz, 1H), 7.53 (t, J = 7.1 Hz, 1H), 7.33 (t, J = 7.1 Hz, 1H), 6.48 (d, J = 4.1 Hz, 2H), 5.92 (s, 2H), 4.07 (s, 1H), 3.86 (s, 3H), 3.65 (s, 2H), 3.48 (t, J = 7.0 Hz, 2H), 3.04 (t, J = 5.6 Hz, 2H), 2.68 (t, J = 5.6 Hz, 2H), 2.63 (t, J = 6.9 Hz, 2H), 1.93–1.85 (m, 4H), 1.74–1.66 (m, 2H), 1.59 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.40, 150.70, 148.84, 147.37, 143.51, 135.00, 134.09, 128.62, 128.25, 123.59, 122.78, 120.22, 115.93, 107.45, 102.16, 101.29, 56.58, 54.01, 49.30, 48.78, 33.95, 29.50, 27.50, 24.86, 23.03, 22.75. Purity: 96.6% by HPLC, HRMS (ESI): calcd for $(M+H)^+$ ($C_{26}H_{31}N_3O_3$) requires *m/z* 434.2444, found 434.2438.

4.4.2. N^1-((7-Methoxybenzo[*d*][1,3]dioxol-5-yl)methyl)-N^5-(1,2,,4-tetrahydroacridin-9-yl)pentane-1,5-diamine (9b)

Intermediate **4a** was treated with **8b** according to general procedure to give the desired product **9b** as light yellow oil (64% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.98–7.84 (m, 2H), 7.52 (t, J = 7.6 Hz, 1H), 7.33–7.28 (m, 1H), 6.49 (s, 2H), 5.90 (s, 2H), 3.86 (s, 3H), 3.65 (s, 2H), 3.45 (t, J = 7.2 Hz, 2H), 3.03 (d, J = 5.6 Hz, 2H), 2.66 (d, J = 5.3 Hz, 2H), 2.58 (t, J = 7.1 Hz, 2H), 1.94–1.79 (m, 4H), 1.74–1.58 (m, 2H), 1.51 (dt, J = 14.3, 7.1 Hz, 2H), 1.40 (dt, J = 9.1, 6.9 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.15, 150.81, 148.80, 147.09, 143.47, 134.64, 134.10, 128.30, 123.56, 122.81, 120.06, 115.69, 107.57, 102.21, 101.25, 56.55, 53.81, 49.22, 48.82, 33.70, 31.53, 29.54, 24.73, 24.61, 22.95, 22.65. Purity: 99.8% by HPLC, HRMS (ESI): calcd for $(M-H)^-$ ($C_{27}H_{33}N_3O_3$) requires *m/z* 446.2444, found 446.2447.

4.4.3. N^1-((7-Methoxybenzo[*d*][1,3]dioxol-5-yl)methyl)-N^6-(1,2,3,4-tetrahydroacridin-9-yl)hexane-1,6-diamine (9c)

Intermediate **4a** was treated with **8c** according to general procedure to give the desired product **9c** as light yellow oil (81% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (dd, J = 13.8, 8.1 Hz, 2H), 7.52 (t, J = 7.0 Hz, 1H), 7.31 (t, J = 7.1 Hz, 1H), 6.49 (d, J = 1.5 Hz, 2H), 5.90 (s, 2H), 3.86 (s, 3H), 3.64 (s, 2H), 3.44 (t, J = 7.2 Hz, 2H), 3.04 (d, J = 6.1 Hz, 2H), 2.67 (t, J = 5.3 Hz, 2H), 2.56 (t, J = 7.2 Hz, 2H), 2.17 (s, 1H), 1.93–1.83 (m, 4H), 1.67–1.57 (m, 2H), 1.53–1.43 (m, 2H), 1.41–1.29 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.36, 150.68, 148.77, 147.44, 143.46, 135.18, 133.99, 128.67, 128.16, 123.50, 122.82, 120.22, 115.84, 107.38, 102.13, 101.23, 56.54, 54.04, 49.37, 49.12, 34.02, 31.68, 29.94, 27.08, 26.83, 24.76, 23.03, 22.77. Purity: 99.8% by HPLC, HRMS (ESI): calcd for $(M-H)^-$ ($C_{28}H_{35}N_3O_3$) requires *m/z* 460.2600, found 460.2600.

4.4.4. N^1-((7-Methoxybenzo[*d*][1,3]dioxol-5-yl)methyl)-N^7-(1,2,3,4-tetrahydroacridin-9-yl)heptane-1,7-diamine (9d)

Intermediate **4a** was treated with **8d** according to general procedure to give the desired product **9d** as light yellow oil (81% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (dd, J = 12.4, 8.3 Hz, 2H), 7.54 (t, J = 7.6 Hz, 1H), 7.33 (t, J = 7.2 Hz, 1H), 6.51 (s, 2H), 5.93 (s, 2H), 3.89 (s, 3H), 3.69 (s, 2H), 3.63 (s, 1H), 3.49 (t, J = 7.2 Hz, 2H), 3.06 (s, 2H), 2.68 (s, 2H), 2.64–2.56 (m, 2H), 2.02 (s, 1H), 1.90 (t, J = 3.1 Hz, 4H), 1.71–1.59 (m, 2H), 1.55–1.46 (m, 2H), 1.41–1.28 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.98, 151.09, 148.84, 146.84, 143.54, 134.24, 134.10, 128.50, 128.09, 123.64, 122.88, 119.93, 115.48, 107.72, 102.39, 101.33, 56.59, 53.62, 49.37, 48.79, 33.48, 31.65, 29.47, 29.19, 27.15, 26.82, 24.71, 22.96, 22.61. Purity: 99.3% by HPLC, HRMS (ESI): calcd for $(M-H)^-$ ($C_{29}H_{37}N_3O_3$) requires *m/z* 474.2757, found 474.2759.

4.4.5. N^1-((7-Methoxybenzo[*d*][1,3]dioxol-5-yl)methyl)-N^8-(1,2,3,4-tetrahydroacridin-9-yl)octane-1,8-diamine (9e)

Intermediate **4a** was treated with **8e** according to general procedure to give the desired product **9e** as light yellow oil (65% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, J = 8.4 Hz, 1H), 7.91 (d, J = 8.4 Hz, 1H), 7.52 (t, J = 7.5 Hz, 1H), 7.31 (t, J = 7.6 Hz, 1H), 6.50 (s, 2H), 5.91 (s, 2H), 3.87 (s, 3H), 3.66 (s, 2H), 3.46 (t, J = 7.2 Hz,

2H), 3.05 (s, 2H), 2.67 (s, 2H), 2.57 (t, J = 7.2 Hz, 2H), 1.94–1.83 (m, 4H), 1.62 (dd, J = 14.4, 7.3 Hz, 2H), 1.53–1.42 (m, 2H), 1.35–1.22 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.21, 150.83, 148.76, 147.26, 143.47, 135.07, 134.01, 128.46, 128.24, 123.50, 122.86, 120.11, 115.66, 107.43, 102.18, 101.23, 56.53, 53.98, 49.40, 49.21, 33.86, 31.69, 29.92, 29.37, 29.24, 27.19, 26.82, 24.72, 23.00, 22.71. Purity: 97.5% by HPLC, HRMS (ESI): calcd for $(M-H)^-$ ($C_{30}H_{39}N_3O_3$) requires m/z 488.2913, found 488.2918.

4.4.6. N^1-((7-Methoxybenzo[*d*][1,3]dioxol-5-yl)methyl)-N^9-(1,2,3,4-tetrahydroacridin-9-yl)nonane-1,9-diamine (9f)

Intermediate **4a** was treated with **8f** according to general procedure to give the desired product **9f** as light yellow oil (63% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, J = 8.4, 1H), 7.90 (d, J = 8.4, 1H), 7.53 (t, J = 7.3, 1H), 7.33 (d, J = 7.4, 1H), 6.50 (s, 2H), 5.92 (s, 2H), 3.88 (s, 3H), 3.67 (s, 2H), 3.47 (t, J = 7.2, 2H), 3.05 (s, 2H), 2.68 (s, 2H), 2.58 (t, J = 7.3, 2H), 1.94–1.83 (m, 4H), 1.69–1.59 (m, 2H), 1.48 (dd, J = 13.8, 7.0, 2H), 1.37–1.24 (m, 10H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.26, 150.85, 148.78, 147.29, 143.50, 134.87, 134.07, 128.49, 128.26, 123.51, 122.86, 120.12, 115.67, 107.48, 102.24, 101.27, 56.54, 53.92, 49.45, 49.16, 33.85, 31.72, 29.87, 29.41, 29.38, 29.26, 27.26, 26.87, 24.74, 23.02, 22.72. Purity: 98.9% by HPLC, HRMS (ESI): calcd for $(M-H)^-$ ($C_{31}H_{41}N_3O_3$) requires m/z 502.3070, found 502.3077.

4.4.7. N^1-(1,2,3,4-Tetrahydroacridin-9-yl)-N^6-(3,4,5-trimethoxybenzyl)hexane-1,6-diamine (9g)

Aldehyde **4b** was treated with **8c** according to general procedure to give the desired product **9g** as light yellow oil (75% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (dd, J = 16.0, 7.8 Hz, 2H), 7.52 (t, J = 7.0 Hz, 1H), 7.31 (t, J = 7.0 Hz, 1H), 6.55 (s, 2H), 3.83 (d, J = 4.3 Hz, 9H), 3.70 (s, 2H), 3.45 (t, J = 7.2 Hz, 2H), 3.04 (d, J = 6.1 Hz, 2H), 2.67 (t, J = 5.3 Hz, 2H), 2.61 (t, J = 7.2 Hz, 2H), 2.30 (s, 1H), 1.93–1.84 (m, 4H), 1.69–1.58 (m, 2H), 1.57–1.45 (m, 2H), 1.42–1.32 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.35, 153.14, 150.69, 147.42, 136.78, 136.21, 128.64, 128.17, 123.50, 122.81, 120.20, 115.83, 104.90, 60.80, 56.07, 54.35, 49.36, 34.00, 31.68, 29.95, 27.10, 26.84, 24.75, 23.02, 22.75. Purity: 99.4% by HPLC, HRMS (ESI): calcd for $(M-H)^-$ ($C_{29}H_{39}N_3O_3$) requires m/z 476.2913, found 476.2919.

4.4.8. N^1-(1,2,3,4-Tetrahydroacridin-9-yl)-N^7-(3,4,5-trimethoxybenzyl)heptane-1,7-diamine (9h)

Aldehyde **4b** was treated with **8d** according to general procedure to give the desired product **9h** as light yellow oil (66% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.97 (d, J = 8.5 Hz, 1H), 7.92 (d, J = 8.5 Hz, 1H), 7.53 (t, J = 7.6 Hz, 1H), 7.32 (t, J = 7.7 Hz, 1H), 6.61 (s, 2H), 5.06 (s, 1H), 3.84 (s, 6H), 3.82 (s, 3H), 3.77 (s, 2H), 3.50 (t, J = 7.2 Hz, 2H), 3.04 (s, 2H), 2.65 (t, J = 7.3 Hz, 4H), 1.99 (s, 1H), 1.87 (s, 4H), 1.73–1.59 (m, 2H), 1.55 (s, 2H), 1.34 (d, J = 15.9 Hz, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 157.13, 153.17, 151.53, 145.85, 137.17, 133.77, 128.78, 127.08, 123.67, 123.03, 119.42, 114.86, 105.53, 60.72, 56.05, 53.28, 49.09, 48.45, 32.78, 31.47, 29.04, 28.77, 27.00, 26.69, 24.57, 22.78, 22.33. Purity: 99.7% by HPLC, HRMS (ESI): calcd for $(M-H)^-$ ($C_{30}H_{41}N_3O_3$) requires m/z 490.3070, found 490.3074.

4.4.9. N^1-(1,2,3,4-Tetrahydroacridin-9-yl)-N^8-(3,4,5-trimethoxybenzyl)octane-1,8-diamine (9i)

Aldehyde **4b** was treated with **8e** according to general procedure to give the desired product **9i** as light yellow oil (83% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, J = 8.5 Hz, 1H), 7.91 (d, J = 8.4 Hz, 1H), 7.52 (t, J = 7.6 Hz, 1H), 7.32 (t, J = 7.6 Hz, 1H), 6.56 (s, 2H), 3.85 (s, 6H), 3.83 (s, 3H), 3.71 (s, 2H), 3.45 (d, J = 7.2 Hz, 2H), 3.05 (s, 2H), 2.68 (s, 2H), 2.62 (t, J = 7.2 Hz, 2H), 1.94–1.84 (m, 4H), 1.69–1.58 (m, 2H), 1.49 (dd, J = 13.1, 6.4 Hz, 2H), 1.36–1.24 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.21, 153.14, 150.82, 147.27, 136.81, 136.17, 128.47, 128.24, 123.50, 122.86, 120.11, 115.67, 104.95, 60.76, 56.04, 54.31, 49.49, 49.40, 33.87, 31.68, 29.96, 29.39, 29.25, 27.22, 26.83, 24.72, 23.00, 22.71. Purity: 98.5% by HPLC, HRMS (ESI): calcd for $(M-H)^-$ ($C_{31}H_{43}N_3O_3$) requires m/z 504.3226, found 504.3226.

4.4.10. N^1-(1,2,3,4-Tetrahydroacridin-9-yl)-N^9-(3,4,5-trimethoxybenzyl)nonane-1,9-diamine (9j)

Aldehyde **4b** was treated with **8f** according to general procedure to give the desired product **9j** as light yellow oil (74% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, J = 8.1 Hz, 1H), 7.90 (d, J = 8.0 Hz, 1H), 7.52 (t, J = 7.1 Hz, 1H), 7.32 (s, 1H), 6.57 (s, 2H), 3.85 (s, 6H), 3.83 (s, 3H), 3.72 (s, 2H), 3.46 (t, J = 7.2 Hz, 2H), 3.05 (s, 2H), 2.68 (s, 2H), 2.65–2.59 (m, 2H), 1.90 (dd, J = 6.2, 3.1 Hz, 4H), 1.69–1.58 (m, 2H), 1.54–1.47 (m, 2H), 1.38–1.27 (m, 10H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.22, 153.15, 150.84, 147.26, 136.84, 135.95, 128.44, 128.23, 123.48, 122.86, 120.10, 115.63, 105.01, 60.76, 56.04, 54.20, 49.40, 33.82, 31.69, 29.87, 29.40, 29.38, 29.24, 27.27, 26.85, 24.71, 22.99, 22.70. Purity: 99.4% by HPLC, HRMS (ESI): calcd for $(M-H)^-$ ($C_{32}H_{45}N_3O_3$) requires m/z 518.3383, found 518.3389.

4.4.11. N^1-(3,4-Dimethoxybenzyl)-N^6-(1,2,3,4-tetrahydroacridin-9-yl)hexane-1,6-diamine (9k)

Aldehyde **4c** was treated with **8c** according to general procedure to give the desired product **9k** as light yellow oil (88% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.92 (dd, J = 12.5, 8.6 Hz, 2H), 7.51 (t, J = 7.6 Hz, 1H), 7.30 (d, J = 7.6 Hz, 1H), 6.87 (s, 1H), 6.80 (q, J = 8.3 Hz, 2H), 3.96 (s, 1H), 3.84 (d, J = 11.2 Hz, 6H), 3.69 (s, 2H), 3.44 (t, J = 6.9 Hz, 2H), 3.04 (s, 2H), 2.66 (s, 2H), 2.58 (t, J = 7.1 Hz, 2H), 2.51 (s, 1H), 1.88 (s, 4H), 1.61 (dd, J = 13.9, 7.0 Hz, 2H), 1.55–1.44 (m, 2H), 1.42–1.29 (m, 4H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.33, 150.70, 148.91, 147.96, 147.39, 132.97, 128.59, 128.17, 123.49, 122.81, 120.19, 115.80, 111.44, 111.04, 55.87, 55.81, 53.80, 49.34, 49.21, 33.94, 31.65, 29.88, 27.07, 26.81, 24.74, 23.01, 22.73. Purity: 98.9% by HPLC, HRMS (ESI): calcd for $(M-H)^-$ ($C_{28}H_{37}N_3O_2$) requires m/z 446.2808, found 446.2811.

4.4.12. N^1-(3,4-Dimethoxybenzyl)-N^7-(1,2,3,4-tetrahydroacridin-9-yl)heptane-1,7-diamine (9l)

Aldehyde **4c** was treated with **8d** according to general procedure to give the desired product **9l** as light yellow oil (65% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.93 (dd, J = 14.0, 8.5 Hz, 2H), 7.52 (t, J = 7.6 Hz, 1H), 7.34–7.30 (m, 1H), 6.91 (s, 1H), 6.84 (d, J = 8.2 Hz, 1H), 6.79 (d, J = 8.1 Hz, 1H), 3.87 (s, 3H), 3.83 (s, 3H), 3.73 (s, 2H), 3.46 (t, J = 7.2 Hz, 2H), 3.05 (s, 2H), 2.67 (s, 2H), 2.61 (t, J = 7.3 Hz, 2H), 1.99 (s, 1H), 1.94–1.82 (m, 4H), 1.69–1.58 (m, 2H), 1.56–1.45 (m, 2H), 1.41–1.25 (m, 6H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.11, 150.92, 148.97, 148.18, 147.06, 131.80, 128.34, 128.27, 123.55, 122.85, 120.52, 120.03, 115.59, 111.67, 111.06, 55.87, 55.83, 53.28, 49.34, 48.79, 33.66, 31.62, 29.32, 29.16, 27.13, 26.79, 24.71, 22.96, 22.65. Purity: 99.9% by HPLC, HRMS (ESI): calcd for $(M-H)^-$ ($C_{29}H_{39}N_3O_2$) requires m/z 460.2964, found 460.2971.

4.4.13. N^1-(3,4-Dimethoxybenzyl)-N^8-(1,2,3,4-tetrahydroacridin-9-yl)octane-1,8-diamine (9m)

Aldehyde **4c** was treated with **8e** according to general procedure to give the desired product **9m** as light yellow oil (69% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.94 (d, J = 8.4 Hz, 1H), 7.90 (d, J = 8.4 Hz, 1H), 7.52 (t, J = 7.3 Hz, 1H), 7.31 (t, J = 7.5 Hz, 1H), 6.88 (d, J = 1.2 Hz, 1H), 6.84–6.76 (m, 2H), 3.87 (s, 3H), 3.83 (s, 3H), 3.71 (s, 2H), 3.45 (t, J = 7.2 Hz, 2H), 3.04 (d, J = 5.7 Hz, 2H), 2.67 (s, 2H), 2.60 (t, J = 7.2 Hz, 2H), 1.95–1.84 (m, 4H), 1.68–1.57 (m, 2H), 1.55–1.44 (m, 2H), 1.36–1.24 (m, 8H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.27, 150.79, 148.93, 147.97, 147.34, 132.95, 128.54,

128.21, 123.50, 122.85, 120.22, 120.15, 115.71, 111.46, 111.03, 55.87, 55.82, 53.77, 49.41, 49.33, 33.91, 31.69, 29.91, 29.38, 29.25, 27.21, 26.82, 24.74, 23.01, 22.73. Purity: 99.0% by HPLC, HRMS (ESI): calcd for $(M-H)^-$ ($C_{30}H_{41}N_3O_2$) requires *m/z* 474.3121, found 474.3123.

4.4.14. N^1-(3,4-Dimethoxybenzyl)-N^9-(1,2,3,4-tetrahydroacridin-9-yl)nonane-1,9-diamine (9n)

Aldehyde **4c** was treated with **8e** according to general procedure to give the desired product **9m** as light yellow oil (70% yield). ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.95 (d, J = 7.9 Hz, 1H), 7.90 (d, J = 7.8 Hz, 1H), 7.52 (t, J = 7.0 Hz, 1H), 7.32 (s, 1H), 6.89 (s, 1H), 6.83 (d, J = 8.1 Hz, 1H), 6.79 (d, J = 8.1 Hz, 1H), 3.87 (s, 3H), 3.84 (s, 3H), 3.71 (s, 2H), 3.45 (t, J = 7.2 Hz, 2H), 3.04 (d, J = 6.0 Hz, 2H), 2.68 (d, J = 5.4 Hz, 2H), 2.64–2.57 (m, 2H), 1.89 (dd, J = 6.3, 3.1 Hz, 4H), 1.68–1.57 (m, 2H), 1.49 (dd, J = 14.0, 7.1 Hz, 2H), 1.38–1.25 (m, 10H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 158.29, 150.79, 148.93, 148.00, 147.36, 132.77, 128.55, 128.20, 123.48, 122.84, 120.26, 120.15, 115.69, 111.49, 111.03, 55.87, 55.81, 53.68, 49.42, 49.26, 33.90, 31.70, 29.84, 29.40, 29.38, 29.24, 27.27, 26.85, 24.73, 23.01, 22.73. Purity: 99.5% by HPLC, HRMS (ESI): calcd for $(M-H)^-$ ($C_{31}H_{43}N_3O_2$) requires *m/z* 488.3277, found 488.3274.

4.5. Biological activity

4.5.1. In vitro inhibition studies on AChE and BuChE

Acetylcholinesterase (AChE, E.C. 3.1.1.7, from *electric eel*), butyrylcholinesterase (BuChE, E.C. 3.1.1.8, from *equine serum*), 5,5'-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent, DTNB), acetylthiocholine chloride (ATC), butylthiocholine chloride (BTC), and tarcine hydrochloride were purchased from Sigma Aldrich. Tacrine and synthesized derivatives were dissolved in DMSO and then diluted in 0.1 M KH_2PO_4/K_2HPO_4 buffer (pH 8.0) to provide a final concentration range.

All the assays were under 0.1 M KH_2PO_4/K_2HPO_4 buffer, pH 8.0, using a Shimadzu UV-2450 Spectrophotometer. Enzyme solutions were prepared to give 2.0 units/mL in 2 mL aliquots. The assay medium contained phosphate buffer, pH 8.0 (1 mL), 50 μL of 0.01 M DTNB, 10 μL of enzyme, and 50 μL of 0.01 M substrate (acetylthiocholine chloride). The substrate was added to the assay medium containing enzyme, buffer, and DTNB with inhibitor after 15 min of incubation time. The activity was determined by measuring the increase in absorbance at 412 nm at 1 min intervals at 37 °C. Calculations were performed according to the method of the equation in Ellman et al.[31] In vitro BuChE assay use the similar method described above. Each concentration was assayed in triplicate.

4.5.2. Kinetic characterization of AChE inhibition

Kinetic characterization of AChE was performed using a reported method.[21] Six different concentrations of substrate were mixed in the 1 mL 0.1 M KH_2PO_4/K_2HPO_4 buffer (pH 8.0), containing 50 μL of DTNB, 10 μL AChE, and 50 μL substrate. Test compound was added into the assay solution and pre-incubated with the enzyme at 37 °C for 15 min, followed by the addition of substrate. Kinetic characterization of the hydrolysis of ATC catalyzed by AChE was done spectrometrically at 412 nm. A parallel control with no inhibitor in the mixture, allowed adjusting activities to be measured at various times.

4.5.3. Molecular modelincg

The crystal structure of the torpedo acetylcholinesterase complexed with tacrine (code ID: 1ACJ) and the human butyrylcholinesterase complexed with echothiophate (code ID: 1POI) were obtained in the Protein Data Bank after eliminating the inhibitor and water molecules. The 3D Structure of **9e** was built and performed geometry optimization by molecular mechanics. Further preparation of substrates included addition of Gasteiger charges, removal of hydrogen atoms and addition of their atomic charges to skeleton atoms, and finally, assignment of proper atomic types. Autotors was then used to define the rotatable bonds in the ligand.

Docking studies were carried out using the AUTODOCK 4.0 program. Using ADT, Polar hydrogen atoms were added to amino acid residues and Gasteiger charges were assigned to all atoms of the enzyme. The resulting enzyme structure was used as an input for the AUTOGRID program. AUTOGRID performed a precalculated atomic affinity grid maps for each atom type in the ligand plus an electrostatics map and a separate desolvation map present in the substrate molecule. All maps were calculated with 0.375 Å spacing between grid points. The centre of the grid box was placed at the bottom of the active site gorge (AChE [2.781 64.383 67.971]; BuChE [112.0 20.0 40.0]). The dimensions of the active site box were set at $50 \times 46 \times 46$ Å.

Flexible ligand docking was performed for the compounds. Docking calculations were carried out using the Lamarckian genetic algorithm (LGA) and all parameters were the same for each docking. To ensure the reliability of the results, the docking procedures were repeated 10 independent times for each compound and the obtained orientations were analyzed.

4.5.4. Inhibition of self-mediated Aβ(1–42) aggregation

In order to investigate the self-mediated Aβ(1–42) aggregation, a thioflavin-T fluorescence assay was performed.[34] Aβ(1–42) peptide (Anaspec Inc) was dissolved in phosphate buffer (pH 7.40, 0.01 M) to obtain a 20 μM solution. Compounds were firstly prepared in DMSO to obtain a 10 mM solution. The final concentration of Aβ(1–42) and inhibitors were 20 μM. After incubated in 37 °C for 48 h, thioflavin-T (5 μM in 50 mM glycine–NaOH buffer, pH 8.00) was added. Fluorescence was measured at 450 nm (λ_{ex}) and 485 nm (λ_{em}). Each inhibitor was run in triplicate. The fluorescence intensities were recorded, and the percentage of inhibition on aggregation was calculated by the following expression: $(1 - I_{Fi}/I_{Fc}) * 100\%$ in which I_{Fi} and I_{Fc} were the fluorescence intensities obtained for absorbance in the presence and absence of inhibitors, respectively, after subtracting the fluorescence of respective blanks.

5. Conflict of interest

We declare that we have no conflict of interest.

Acknowledgements

We thank the Natural Science Foundation of China (Grants U0832005, 90813011, 20772159, 81001400), and the Science Foundation of Guangzhou (2009A1-E011-6) for financial support of this study.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.12.022.

References and notes

1. Kung, H. F.; Lee, C. W.; Zhuang, Z. P.; Kung, M. P.; Hou, C.; Plössl, K. *J. Am. Chem. Soc.* **2001**, *123*, 12740.
2. Förstl, H.; Kurz, A. *Eur. Arch. Psychiatry Clin. Neurosci.* **1999**, *249*, 288.
3. Fernandez-Bachiller, M. I.; Perez, C.; Campillo, N. E.; Paez, J. A.; Gonzalez-Munoz, G. C.; Usan, P.; Garcia-Palomero, E.; Lopez, M. G.; Villarroya, M.; Garcia, A. G.; Martinez, A.; Rodriguez-Franco, M. I. *ChemMedChem* **2009**, *4*, 828.
4. Yankner, B. A.; Dawes, L. R.; Fisher, S.; Villa-Komaroff, L.; Oster-Granite, M. L.; Neve, R. L. *Science* **1989**, *245*, 417.

5. Masters, C. L.; Simms, G.; Weinman, N. A.; Multhaup, G.; McDonald, B. L.; Beyreuther, K. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 4245.
6. Cummings, J. L.; Doody, R.; Clark, C. *Neurology* **2007**, *69*, 1622.
7. Cavalli, A.; Bolognesi, M. L.; Minarini, A.; Rosini, M.; Tumiatti, V.; Recanatini, M.; Melchiorre, C. *J. Med. Chem.* **2008**, *51*, 347.
8. Rizzo, S.; Riviere, C.; Piazzi, L.; Bisi, A.; Gobbi, S.; Bartolini, M.; Andrisano, V.; Morroni, F.; Tarozzi, A.; Monti, J. P.; Rampa, A. *J. Med. Chem.* **2008**, *51*, 2883.
9. Ibach, B.; Haen, E. *Curr. Pharm. Des.* **2004**, *10*, 231.
10. Mesulam, M. M.; Guillozet, A.; Shaw, P.; Levey, A.; Duysen, E. G.; Lockridge, O. *Neuroscience* **2002**, *110*, 627.
11. Yankner, B. A.; Duffy, L. K.; Kirschner, D. A. *Science* **1990**, *250*, 279.
12. Lorenzo, A.; Yankner, B. A. *Proc. Natl. Acad. Sci. U.S.A.* **1994**, *91*, 12243.
13. Thorsett, E. D.; Latimer, L. H. *Curr. Opin. Chem. Biol.* **2000**, *4*, 377.
14. Wolfe, M. S.; Citron, M.; Diehl, T. S.; Xia, W.; Donkor, I. O.; Selkoe, D. J. *J. Med. Chem.* **1998**, *41*, 6.
15. Yang, F.; Lim, G. P.; Begum, A. N.; Ubeda, O. J.; Simmons, M. R.; Ambegaokar, S. S.; Chen, P. P.; Kayed, R.; Glabe, C. G.; Frautschy, S. A.; Cole, G. M. *J. Biol. Chem.* **2005**, *280*, 5892.
16. Lim, G. P.; Chu, T.; Yang, F.; Beech, W.; Frautschy, S. A.; Cole, G. M. *J. Neurosci.* **2001**, *21*, 8370.
17. Howlett, D. R.; Perry, A. E.; Godfrey, F.; Swatton, J. E.; Jennings, K. H.; Spitzfaden, C.; Wadsworth, H.; Wood, S. J.; Markwell, R. E. *Biochem. J.* **1999**, *340*, 283.
18. Ono, M.; Kung, M.-P.; Hou, C.; Kung, H. F. *Nucl. Med. Biol.* **2002**, *29*, 633.
19. Rosini, M.; Simoni, E.; Bartolini, M.; Cavalli, A.; Ceccarini, L.; Pascu, N.; McClymont, D. W.; Tarozzi, A.; Bolognesi, M. L.; Minarini, A.; Tumiatti, V.; Andrisano, V.; Mellor, I. R.; Melchiorre, C. *J. Med. Chem.* **2008**, *51*, 4381.
20. Camps, P.; Formosa, X.; Galdeano, C.; Munoz-Torrero, D.; Ramirez, L.; Gomez, E.; Isambert, N.; Lavilla, R.; Badia, A.; Clos, M. V.; Bartolini, M.; Mancini, F.; Andrisano, V.; Arce, M. P.; Rodriguez-Franco, M. I.; Huertas, O.; Dafni, T.; Luque, F. J. *J. Med. Chem.* **2009**, *52*, 5365.
21. Tang, H.; Ning, F. X.; Wei, Y. B.; Huang, S. L.; Huang, Z. S.; Chan, A. S.; Gu, L. Q. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 3765.
22. Pan, L.; Tan, J. H.; Hou, J. Q.; Huang, S. L.; Gu, L. Q.; Huang, Z. S. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3790.
23. Wang, B.; Mai, Y. C.; Li, Y.; Hou, J. Q.; Huang, S. L.; Ou, T. M.; Tan, J. H.; An, L. K.; Li, D.; Gu, L. Q.; Huang, Z. S. *Eur. J. Med. Chem.* **2010**, *45*, 1415.
24. Hung, T. M.; Na, M.; Min, B. S.; Ngoc, T. M.; Lee, I.; Zhang, X.; Bae, K. *Arch. Pharm. Res.* **2007**, *30*, 685.
25. Elsinghorst, P. W.; Tanarro, C. M.; Gutschow, M. *J. Med. Chem.* **2006**, *49*, 7540.
26. Ucar, G.; Gokhan, N.; Yesilada, A.; Bilgin, A. A. *Neurosci. Lett.* **2005**, *382*, 327.
27. Nadri, H.; Pirali-Hamedani, M.; Shekarchi, M.; Abdollahi, M.; Sheibani, V.; Amanlou, M.; Shafiee, A.; Foroumadi, A. *Bioorg. Med. Chem.* **2010**, *18*, 6360.
28. Tomita, M.; Aoyagi, Y. *Chem. Pharm. Bull.* **1968**, *16*, 523.
29. Carlier, P. R.; Han, Y. F.; Chow, E. S.; Li, C. P.; Wang, H.; Lieu, T. X.; Wong, H. S.; Pang, Y. P. *Bioorg. Med. Chem.* **1999**, *7*, 351.
30. Zhou, J. J.; Huang, H.; Xie, S. Q.; Wang, Y. X.; Zhao, J.; Wang, C. J. *Chin. Chem. Lett.* **2008**, *19*, 99.
31. Ellman, G. L.; Courtney, K. D., Jr.; Andres, B.; Featherstone, R. M. *Biochem. Pharmacol.* **1961**, *7*, 88.
32. Morris, G. M.; Goodsell, D. S.; Halliday, R. S.; Huey, R.; Hart, W. E.; Belew, R. K.; Olson, A. J. *J. Comput. Chem.* **1998**, *19*, 1639.
33. DeLano, W. L. *The PyMOL Molecular Graphics System*; DeLano Scientific: San Carlos, CA, 2002.
34. Bartolini, M.; Bertucci, C.; Bolognesi, M. L.; Cavalli, A.; Melchiorre, C.; Andrisano, V. *Chembiochem* **2007**, *8*, 2152.
35. Schneiders, G. E.; Stevenson, R. *J. Org. Chem.* **1981**, *46*, 2969.